Standard Operating Procedure for the Analysis of Total Phosphorus and Total Nitrogen in Water from an Alkaline Persulfate Digest

1.0 Scope and Applicability

This method is applicable to the determination of total phosphorus and total nitrogen in water. The MDL for phosphorus and TKN are 0.004 mg P/L and 0.015 mg N/L. The MDLs were developed using spikes in reagent grade (17 meg-ohm) water to which 1.309 ml of 11 N sulfuric acid was added to simulate field preserved samples. The spiked samples were then digested in the block digestor and a blank correction was made. Total phosphorus may also be measured by using the ammonium persulfate digest method (SOP I-1-12).

2.0 Summary of Method

Twenty milliliters of a sample are placed into a 25 X 150 mm culture tube with 5 ml of a digestion solution containing sodium hydroxide and potassium persulfate. The tube is sealed with a screw cap and placed into a block digestor at 150 deg C for 15 minutes. The tube is then cooled, opened and 0.5 ml of 11 N H_2SO_4 containing additional potassium persulfate is added. The sample tube is resealed and placed back into the block digestor for 30 minutes. The tube is then cooled and the contents are ready for analysis of total phosphorus and total nitrogen by flow injection analysis.

In this TKN digest, potassium persulfate in an alkaline environment converts all forms of nitrogen containing compounds to the nitrate form. The oxygen for this oxidation comes from the reaction in equation one:

eq. 1. $K_2S_2O_8 + H_2O = 2KHSO_4 + 1/2O_2$
eq. 2. $KHSO_4 + NaOH = Na^+ + K^+ + SO_4^{2-} + H_2O$

The sodium hydroxide present must be sufficient to neutralize the hydrogen ions released in equation two and maintain an alkaline environment during the nitrogen digestion step. As one mole of potassium persulfate produces two moles of hydrogen ions ; an approximately equal molar ratio of sodium hydroxide and potassium persulfate at the start of the digestion should produce an acid environment at the end of the digestion when all the sodium hydroxide is consumed. The Nitrate produced by this digestion is further reduced to NO_2 almost quantitatively in the presence of cadmium when analyzed online with the FIA. This method uses commercially available Cd granules treated with copper sulfate and packed in a glass column. The NO_2 produced is determined by diazotizing with sulfanilamide and

coupling with N- (1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically at 520 μm by FIA.

This is a true total nitrogen analysis. This compares in the final result to the historic Total Kjeldahl Nitrogen analysis in which all organic nitrogen was converted to ammonia nitrogen and all nitrate nitrogen was reduced to ammonia. Unlike the historic TKN analysis which used mercury as a catalyst, a sulfuric acid digest and chromium to reduce nitrate, no heavy metals are used in this digestion procedure. To compare this result to the acid digested TKN result previously determined (EPA method 351.2), it will be necessary to subtract the nitrate contribution. For the alkaline persulfate digestion of nitrogen to proceed to completion the acid present in the preserved samples must not be so high as to completely neutralize the sodium hydroxide in the digestion solution before the digestion is complete. The range for this procedure is 0.01 to 2.5 mg nitrate/L.

Phosphorous occurs in several forms: ortho, poly and organically bound. Further classification can be made on whether it is dissolved. This last classification is defined as passing through a 0.45 micron filter. The various forms and classifications are outlined in section 17.0. All forms of phosphorus are converted to the ortho form in this procedure in the presence of potassium persulfate and an acid background. This acid environment must be present at the end of the nitrogen digestion. If too much acid is added in the field to preserve the samples or too much sodium hydroxide is added to neutralize the acid then the conditions will not be proper for the phosphorus portion of the digestion and results will be in error. Differences in pH of samples at this step in the analysis result in a positive bias for phosphate of approximately 0.1 ppm or higher. To ensure proper pH and available persulfate, additional sulfuric acid containing potassium persulfate is added prior to the phosphorus digestion step.

Some departure from the Standard Methods (SM), 19th Ed. "Proposed" Persulfate Method 4500-N_{org} D has been made. Standard Methods does not attempt to analyze acid preserved samples. This has been accomplished by neutralizing the preserving acid prior to alkaline digest. Additional sodium hydroxide has been added to the digestion solution to neutralize the preserving acid added in the field which was 2 ml of 1:5 sulfuric acid added to a 500 ml plastic container. Because the phosphorus results were found to be inconsistent and positively biased after the alkaline digest step due to differences in sample make up, which in turn altered the pH, additional acid is added after the nitrate conversion step but, before the phosphorus digestion takes place. The buffer added after the phosphorus digest step in SM has also been eliminated. The determinative step in the Nitrogen analysis used a strong buffer and the Phosphorus determinative step uses an acid environment making the SM buffer pointless. A separate line has been added to the FIA nitrate manifold board in order

to neutralize the extra acid added for the phosphorus digestion step. The 100 to 110 deg C digestion temperature recommended in SM was found to be low given the geometry of our block and tubes. The optimum temperature was found to be 150 deg C. A set of seven samples of a 2.5 mg/L glutamic acid solution should be digested for 15 min at increasing temperatures starting at 110 deg C until the optimum temperature is found. Instead of 30 min for digestion in SM for the nitrogen step, 15 minutes was found to be adequate. This confirms what Nydahl (see 16.6) found in his studies. The range for the analysis of Phosphorus is 0.004 to 1 mg/L.

3.0 Definitions

The definitions below are specific to this method, but have been conformed to common usage as much as possible.

FIA	Flow injection analysis (a colorimetric procedure)
mg N/L	milligrams Nitrogen per Liter
ppm	parts per million
MDL	Method Detection Level
LIMS	Laboratory Information Management System
SRM	Standard Reference Material from National Institute of Standards and Technology
TKN	Total Kjeldahl Nitrogen
SM	Standard Methods For The Examination of Water and Wastewater
C of A	Certificate of Analysis

4.0 Interferences

In the normal analysis of nitrate plus nitrite the efficiency of the cadmium column would be of concern because, any nitrite present would be read without any conversion loss due to column efficiency. It was initially thought that column efficiency would not play a significant role in the analysis of total nitrogen as all nitrite is converted to nitrate during the digestion but, experience has shown that poor recovery of check samples can be related to column efficiency. Column efficiency should be monitored. Recharging the column with copper sulfate or column replacement should follow the procedure used for nitrate analysis. In general the column efficiency should be above 90%. If treatment (sec. 11.3.4) fails to bring the efficiency up to 85%, the column should be replaced.

Samples to be run should be free of suspended matter as this will hang up in the column used to convert nitrate to nitrite and shift window timing. Colored samples which absorb at 520 nm will also interfere.

Contaminants in reagent grade chemicals are a problem. The ammonium chloride buffer made from NH_4Cl and NaOH tends to produce a slight pink background color, after passing over the cadmium column, suggesting NO^-_3 contamination. Ammonium chloride buffer made from NH_4OH and HCl does not have as pink a color after passing through the cadmium column. This is the preferred method of preparation for the nitrate buffer.

Several brands of potassium persulfate have been found to contain nitrogen compounds. The potassium persulfate from Baker (J.T.Baker #3239-01) has been found to be relatively free of nitrogen and trace amounts of phosphorus. Considerable improvements can be made by ordering this chemical after first checking the Certificate of Analysis (C of A) for each lot available. Lots containing phosphorus at < .2 ppm can be found, typically .5 ppm phosphorus is available. Lots containing Nitrogen at 0.001% (10 ppm) may be better than other brands but, this still leaves a lot to be desired. These levels, coupled with nitrogen and phosphorus contamination in sodium hydroxide, will translate into a phosphorus blank value of about 20 ppb and about 100 ppb for nitrogen.

Samples containing bromide have been found to lead to serious errors in phosphate results. This is because the bromide ion is converted to elemental bromine very quickly in an acid environment using up the persulfate reagent. Bromide levels of up to 500 ppm have no effect on glutamic acid conversion but, bromide levels of 100 ppm will reduce nicotinic acid conversion to nitrate by 13%. Nitrogen recovery is apparently related to competing reaction rates between nitrogen sources and the bromide ion in an alkaline environment. Samples to be analyzed for nitrogen which contain bromide should use the acid TKN procedure. Samples to be analyzed for Phosphorus which contain bromide should be analyzed using the acid persulfate digest procedure which contains larger quantities of persulfate (EPA method 365.3). Double peaks may be observed during the colorimetric step for phosphorus analysis following method 365.3, usually associated with very low phosphorus levels. This can be corrected by sparging out the dissolved bromine gas with helium for about 10 min.

Trace amounts of nitrogen and phosphorus compounds have been found in reagent grade Sodium Hydroxide. Sodium Hydroxide pellets from Mallinckrodt (#7708) were found to have lower levels of nitrogen and phosphorus. Order by lot number after first checking the C of A.

Samples which contain Phenol at a concentration above 0.2 mg/L will negatively interfere with the nitrate conversion. At 0.5 mg/L of phenol a 10 ppm nitrogen sample (glutamic acid) can give an apparent nitrogen level of almost 0 ppm following the digestion. There are other phenol related compounds, not fully investigated, which will impact this analysis more severely.

Nydahl (see 16.6) suggests that samples high in COD (Chemical Oxygen Demand) will also interfere with the analysis. Using spikes of potassium acid phthlate into synthetic samples containing nicotinic acid a recovery loss of 2% was observed for a COD value of 10 ppm and a recovery loss of 5% was observed for a COD value of 50 ppm. Samples high in COD should be diluted or run using the acid TKN procedure, (EPA method 351.1).

Although not an interference the efficiency of the digestion is directly related to the results. Adenosine 5'-triphosphate is a fairly hard compound to digest and thus provides a good measure of digestion efficiency. Long term results have shown that phosphorus is recovered at about 90% (see section 17 for control charts). The recovery of nitrogen for this compound is not quite as good. Average recovery was measured at 77% over this same period. A closer look at this compound shows that there is one amine nitrogen and three nitrogen atoms in ring structures of approximately the same type and one nitrogen atom in a 5 member ring bonding a second five member ring. This last nitrogen which has three carbon atoms singly bonded to it, is suspected as not being digested appreciably. If this is the case and the percent recovery is then based upon four nitrogen atoms, the percent recovery would rise to 96%. There is evidence to support this in Nydahl's paper. He digested over 40 different compounds containing nitrogen and found only a few with very poor recoveries. There were four with ring structures and recoveries of less than 70%: Creatinine 67.9%, Antipyrine 45%, Benzotriazole 34.7% and Methyl Orange 33.6%. If we assume that the N:N double bond in methyl orange could not be broken by this digestion and recalculated the recovery based upon the amine nitrogen, the recovery would be 100.8%. Antipyrine and Creatinine both have one nitrogen atom in a similar configuration to Adenosine 5'-triphosphate. If we likewise assume that this nitrogen is not digestible and recalculate Nydahl's recoveries they will be 90% for Antipyrine and 101.8% for Creatinine. Thus it would appear that some forms of nitrogen for example: N:N (nitrogen double bonded to nitrogen) and NC_3 (nitrogen singly bonded to three carbons in a five member ring may not be digestible by this method.

5.0 Safety:

WARNING This method uses Cadmium granules. Cadmium is toxic and carcinogenic. Wear latex gloves when copperizing the granules. All waste Cadmium will be retained in a 200 ml waste bottle and held for proper disposal.

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS's) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies:

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

Lachat QuikChem 8000 Flow Injection Analyzer

6.1 96 position random access sampler

6.2 Watson Marlow proportioning pump (set at 35, 12.2 rpm)

6.3 Injection module (2)

6.4 Colorimeter with:
10 mm flow cell, 520 nm interference filter, and 190 cm of 0.022 inch dia. loop (NO_3)
10 mm flow cell, 880 nm interference filter, and 190 cm of 0.22 inch dia. loop (PO_4)

6.5 Nitrate manifold board, modified

6.6 Phosphorus manifold board

6.7 Heater set to 37 deg C for PO4, 175 cm (0.032 inch I.D.) tubing

6.8 Cadmium column (250 X 3 mm glass column sold by Chrom Tech, Apple Valley, MN. as part # OM-6324)

6.9 Gateway 2000 P5-60 computer

6.10 NEC Multisync 3FGe Monitor

6.11 Omnion Software (ver. 2.0 Jan 99)

6.12 HP 8150 printer

Lachat 46 place digestion block

7.0 Reagents and Standards:

All reagents are ACS Reagent grade or higher.

7.1 Nitrate Reagents

7.1.1 Ammonium chloride buffer (alternative recipe):
To 3 L of reagent water in a 4 L beaker add 420 mL of concentrated HCl, 390 mL of concentrated ammonium hydroxide (NH_4OH) and 4.0 g of EDTA. Dissolve all of the above and bring the solution to a volume of 4 L. Degas with helium.

7.1.2 Color Reagent:
To approximately 1600 mL of reagent water add 200 mL of conc. H_3PO_4. Add 80 g of sulfanilamide and dissolve completely. Dissolve 2.0 g N-1-Naphthylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2CH_2 2HCl$) dilute to two liters. Filter through a 0.45 micron filter. (There appears to be a lot of residue in this solution.) Degas with helium. Store in a dark bottle. This reagent is stable for three to four weeks.

7.1.3 Sodium Hydroxide (0.23 N)
Dissolve 9.35 g Sodium hydroxide (NaOH) in 500 ml of reagent water using a 1 L beaker. Dilute to the 1 L mark and degas with helium.

7.1.4 Granulated Cadmium:
40-60 mesh (E.M. Laboratories, Inc. 500 Exec. Blvd. Elmsford, NY 10523, Cat 2001-2 Cadmium, Coarse Powder).

7.1.5 Line cleaning solution:
Contrad 70 (Fisher Scientific, Pittsburgh Pa. [Cat.# 04-355-1]) has been found to clean very well at about a 1 to 6 dilution.

7.1.6 Dilute hydrochloric acid: (6 N)
Dilute 50 mL concentrated HCl to 100 mL with reagent water.

7.1.7 Copper sulfate solution 2%
Dissolve 20 g of ($CuSO_4 5 H_2O$) in 500 mL of reagent water and dilute to 1 liter.

7.2 Digestion solution
Dissolve 10.48 g (6 g for digestion, 4.48 g for neutralization) of sodium Hydroxide (NaOH) and 42 g of potassium persulfate ($K_2S_2O_8$ - J.T. Baker #3239-01) in about 900 ml of reagent water. When dissolved bring to a final volume of 1 L.

7.3 Phosphorus reagents

7.3.1 Stock Ammonium Molybdate Solution
In a 1L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}•4H_2O$] in approximately 800 ml of reagent water. Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

7.3.2 Stock Antimony Potassium Tartrate Solution
In a 1L beaker, dissolve 3.0 g antimony potassium tartrate [potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6•1/2H_2O$] in approximately 800 ml reagent water. Dilute to 1L and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

7.3.3 Molybdate Color Reagent
In a 1L beaker, add about 500 ml of reagent water and 20 ml of concentrated H_2SO_4. Stir until it cools then add 213 ml of stock ammonium molybdate solution, then 72 ml of stock antimony potassium tartrate solution. Dilute to the 1 L mark and stir until mixed. Degas with Helium. If the solution is slightly colloidal or yellow add additional sulfuric acid with stirring until it clears. If the solution is blue, discard it and prepare fresh stock solutions of 7.3.1 and 7.3.2. Remake the molybdate color reagent with the new stock reagents.

7.3.4 Ascorbic acid

In a 1L beaker dissolve 60.0 g ascorbic acid in about 900 ml of reagent water. When dissolved bring to a volume of 1L. Degas with Helium. Add 1.0 g of

sodium dodecyl sulfate [$CH_3(CH_2)_{11}OSO_3Na$]. Mix with a stir bar. Prepare fresh weekly, more frequently if problems arise.

7.3.5 11 N Sulfuric Acid (H_2SO_4)
Add 305 ml of concentrated sulfuric acid, to about 600 ml of reagent water with stirring in a large beaker. The beaker should be surrounded by an ice bath to reduce the heat. When cool adjust the final volume to 1 liter with reagent water.

7.3.6 11 N Sulfuric Acid (H_2SO_4) with potassium persulfate
Place 11.5 g of potassium persulfate in a 500 ml beaker and add 500 ml of 1l N sulfuric acid. Stir until dissolved.

7.3.7 Phosphate Carrier
0.231 N H_2SO_4
Dilute 42 ml of 11 N Sulfuric acid to 2 liters with reagent water. Degas with Helium.

7.3.8 Nitrate Carrier
Use degassed reagent water.

7.4 Standards:

7.4.1 Stock Nitrite Standard (100 ppm N/L as NO_2).
Dissolve 0.4926 g of sodium nitrite ($NaNO_2$) reagent grade chemical, in approximately 800 ml reagent water and dilute to 1 liter using a volumetric flask. Prepare the nitrite stock solution about every two weeks and store in the refrigerator as it is not stable.

7.4.2 Stock Nitrate Standard (1000 ppm N/L as NO_3)
Dry potassium nitrate (KNO_3 - Fisher Reagent Grade), for 24 hours at 105 ^oC. Store in a desiccator until ready for use. Dissolve 7.2674 g KNO_3 containing 13.76% N, or equivalent nitrate standard, in 800 mL of reagent water in a 1L volumetric flask. Add 2 mL chloroform and bring to a volume of 1L. Store in a dark bottle. The reagent is stable for six months.

7.4.3 Combined Spike Solutions (200 ppm N and 100 ppm P)
Dissolve 0.4398 g KH_2PO_4 and 1.4535 g KNO_3 in 800 mL of reagent water in a 1L volumetric flask. Bring to 1 L volume. Mix and place in a plastic

bottle and store in the refrigerator. Use 0.1 ml for a spike of 1 ppm N and 0.5 ppm P.

7.4.4 Working Nitrate Standard (100 ppm N/L as NO_3)
Pipette 20 ml of the stock nitrate standard (7.4.2) into a 200 ml volumetric flask, and bring to final volume with deionized water. Invert the flask ten times to mix and store in a 200 ml plastic bottle in the refrigerator. Replace monthly.

7.4.5 Stock Phosphorus standard (100 mg P/L)
Dissolve .4398 g of KH_2PO_4 (SRM 200),22.74% P, previously dried at 105 deg C for 2 hours, in 500 ml of reagent water in a 1L volumetric flask and dilute to 1 L.

7.4.6 Low Level Stock Standard A (5 mg N/L, 2 mg P/L)
Pipette 10 mL of nitrate standard (7.4.4) and 4 mL of phosphate standard (7.4.5) into a 200 mL volumetric flask and dilute to 200 mL. Mix and store in a 200 mL plastic bottle in a refrigerator. Replace monthly

7.4.7 Low Level Stock Standard B (7.8431 mg N/L, 3.1372 mg P/L)
Pipette 15.686 ml of 100 mg N/L (7.4.4) and 6.275 ml of 100 mg P/L (7.4.5) into a 200 ml volumetric flask. Dilute to the mark with reagent water. Mix and store in a 200 ml plastic bottle in a refrigerator. Replace monthly.

7.4.8 Calibration Standards, Internal
Combination nitrate and phosphorus standards are prepared by diluting the 100 mg nitrate/L standard (7.4.4) and the 100 mg phosphorus /L standard (7.4.5) into a 500 ml volumetric flasks according to the following table. Standards 2 and 3 use the Low Level Stock standard A (7.4.6).

level	ml NO3	ml PO4	mg N & P/L
7	12.5	5	2.5/1.0
6	6.25	2.5	1.25/0.5
5	.5	.25	0.5/0.25
4	.25	0.1	.25/0.10
3	5 ml LLS A	-	.05/.02
2	1 ml LLS A	-	.01/.004
1	0	0	0

Twenty milliliters of these standards are carried thru the digestion like samples.

7.4.9 Calibration Standards, External

External non-digested standards may be prepared and run against digested samples. Digest a 2.5 ppm N/1.0 ppm P standard for an internal check

It was found that the external standards containing the digestion mixture did not work well. Part of the problem was the variation between different batches of digestion solution and that used in the standards; deterioration of the digestion solution over time and more importantly the fact that the undigested persulfate solution reacts with the nitrate color reagent and/or the cadmium column to give a positive bias to the standards which in turn gave a negative bias to the sample results. External standards must be made up without the digestion solution.

The working standards are prepared by placing the ml of LLS B standard (7.4.7) listed below into a 200 ml volumetric flask and adding 3.92 ml of 11N H_2SO_4 (7.3.5). Bring to the volume mark with reagent water and mix. Transfer to a 200 ml plastic bottle and refrigerate.

		Effective Concentration	
Level	ml of LLS B	mg N/L	mg P/L
7	50	2.5	1.0
6	25	1.25	0.5
5	10	0.5	0.2
4	4	0.2	0.08
3	1	0.05	0.02
2	0.2	0.01	0.004
1	0	0	0

7.5 Check Samples

Check samples to check the digestion should include one or more of the following: Nicotinic acid, Glutamic acid or Adenosine 5'-triphosphate. It is recommended that the Adenosine 5'-triphosphate be included with other checks.

7.5.1 Stock Adenosine 5'-triphosphate, disodium salt hydrate (Aldrich A26209) 99%pure. (100 ppm P, 76.1 ppm N)

Dissolve 0.5991 g in reagent water in a 1 L volumetric flask. Dilute 1 to 100 and use as a check. Theoretical values for the dilution are 0.761 ppm N and 1.0 ppm P.

7.5.2 Stock Glutamic acid monosodium salt (100 ppm)
Dissolve 1.3360 g of glutamic acid $HO_2CCH_2CH_2CH(NH_2)CO_2Na$, FW 187.13 (7.485% N) in 1L of reagent water. Dilute 1:40 and use as a check sample. Theoretical value for this dilution is 2.5 ppm N.

7.5.3 Quality Control Sample
An external check sample such as an EPA Water Pollution sample or other external check with known values for phosphorus and nitrogen.

8.0 Sample Collection, Preservation and Storage

Samples for nutrient analysis (PO_4^{-3}, TKN, NO_3^- and NH_4^+) should be field preserved with 2 ml of 1:5 sulfuric acid per 500 ml of sample. This will preserve the sample to a pH value of 2 or slightly below. Samples with excessive sulfuric acid will not be neutralized properly and may not yield reliable results.

CAUTION: No samples preserved with mercuric chloride should be run as they will degrade the cadmium column.

9.0 Quality Control

Each set of 10 samples or less will include 1 check sample, 1 duplicate and 1 spiked sample. Duplicate, spike and check samples must be within acceptable limits. Check samples are prepared according to section 7.5. A duplicate sample is one which has a separate digestion from the original. The spiked sample is a duplicate digested sample which has been fortified with both analytes at the concentrations of 1mg/L N in the nitrate form and 0.5 mg/L of phosphorus in the ortho form. Each set of 30 samples will include a laboratory fortified blank, LFB, which must run within $\pm$ 10% of its true value. The LFB is prepared by adding the spike solution to reagent water, which has been preserved with 1.309 ml of 11 N sulfuric acid per 500 ml of water similar to samples, and caring it through the digestion like a sample.

Following the standardization for (nitrate plus nitrite), an analysis for a nitrite and a nitrate standard of equal concentration (about 1 mg N/L) will be run. This ratio should show 85% or better on conversion of nitrate to nitrite. Below this level the Cadmium column should be recharged or exchanged with a freshly packed one.

If external standards are used the mean of seven or more blanks should be used to correct for the phosphorus and nitrogen contamination present in the digestion solution. A blank correction will need to be made for each new lot of chemicals used in the digestion solution.

10.0 Calibration and Standardization:

Standardization is achieved using Lachat's Omnion software ver 2.0. Typical calibration curves are displayed in section 17.0. The number of standards and their levels are listed in section 7.4.7 and 7.4.8. The curve is not forced through zero. The curve fit is almost linear. A second or third order fit improves the bias on the lower end of the calibration curve and will run with a correlation coefficient of 0.995 or better. Failure at this point, that can't be corrected by deleting an outlier will require a re-standardization or preparation of new standards. All data stored with the Omnion software used on the QuikChem 8000 provides for imbedding of the calibration curve in the data file. The electronic data file including the chromatograms can be retrieved and reviewed. The file can be reprocessed with new parameters but the original data will remain unchanged.

11.0 Procedures:

There are two steps involved with PO_4 and TKN(NO_3) analysis. These are: preparation and digestion in the block digestor and the color development, detection and analysis on the Lachat FIA.

11.1 Digestion Procedure

11.1.1 Both samples and standards may be carried through the block digestion. If external standards are used at least one standard must be digested and run against the external standards.

11.1.2 Add 20 ml of sample, using a 20 ml syringe and a number 14 square tipped needle, to each (25 X 150 mm) tube using the work list generated from the LIMS.

11.1.3 If the ammonia and nitrate values for the sample are available and they total over the 2.5 ppm (top standard) use an aliquot which would place the result at mid range. For example on a sample running 6 ppm NH_3 and 4ppm Nitrate, (10 ppm total N) use a 2 ml aliquot and 18 ml of reagent water (a 1:10 dilution). Alternatively prepare a 1:10 dilution using a 100 ml flask and place 20 ml of this dilution into the digestion tube.

11.1.4 Add the spikes (0.1 ml) to the samples and LFB, and check samples to the set as laid out in the work list.

11.1.5 Add 5 ml of digestion solution (7.2), using the a repipettor, to each sample tube and QC sample.

11.1.6 Tighten the caps on the tubes, shake to mix.

11.1.7 Place the tubes into the preheated block at 150 deg C.

11.1.8 After the digestion step is completed, 15 min, remove the tubes and place them into a tube rack, carefully, one at a time using a paper towel.
Warning: After the digestion step is completed these tubes are hot and contain At least 20 pounds per square inch pressure. If one is dropped on the floor it will explode with broken glass being projected everywhere.

11.1.9 The tubes may be cooled within 30 minutes by placing the rack with the tubes into a refrigerator.

11.1.10 When tubes can be safely handled by hand, unscrew the cap and add .5 ml of 11 N sulfuric acid solution containing potassium persulfate (7.3.6).

11.1.11 Re-cap the tube and shake to mix the sample.

11.1.12 Place the tubes back into the block for another 30 min.

11.1.13 Remove the tubes and cool.
Warning: After the digestion step is completed these tubes are hot and contain pressure. They will explode if dropped on the floor.

11.1.14 Shake the tubes to mix and pour the contents into autosampler tubes. Analyze on the FIA. Tubes may also be held for several days and perhaps longer, if capped or covered tightly and placed in a refrigerator.

11.2 Automated Colorimetric Procedure

11.2.1 Place the 520 nm filter into the colorimeter and install the 190 cm sample loop on channel #1. Install the Nitrate manifold board, run feed lines through the pump cassette, connect pump tubes to the manifold according to the

diagram (Section 17.0), and snap the pump tubes into place. Place the 880 nm filter into the colorimeter and install the 190 cm loop on channel #2. Install the Phosphorus manifold board and connect all pump tubes according to the diagram. Turn on the pump. Place all feed lines into reagent water. Check for leaks.

11.2.2 Place all feed lines into their proper reagent containers. Allow the lines to fill. Install the Cd column into the Nitrate manifold board.

11.2.3 When samples are loaded into the autosampler, load the proper method and tray on the computer: "TNO_3_PO_4 .met" and "TNO3_PO4.tra" Start the analysis.

11.2.4 Check the Standardization and the column efficiency before continuing the run. See section 10.0.

11.2.5 If the standardization passes, observe that the check samples and LFB are running at acceptable levels before continuing.

11.2.6 Instrument set up parameters

	Nitrogen	Phosphorus
Chemistry	Direct	Bipolar
Injection to peak start	40	30
Peak base width	95	70
% width tolerance	10	15
Threshold	1595	1500
Method cycle period	100	100
Probe in sample	26	26
Sample reaches 1st valve	20	20
Load period	24	24

11.3 Cadmium Preparation

WARNING: Cadmium is toxic and carcinogenic. Wear latex gloves when copperizing the granules. The cadmium granules are cleaned with dilute HCl and copperized with a 2% solution of copper sulfate as follows:

11.3.1 Wash the cadmium with HCl and rinse with reagent water. The color of the cadmium so treated should be silver.

11.3.2 Swirl 10-20 g of cadmium in 100 mL portions of a 2% solution of copper sulfate for five minutes or until the blue color partially fades, decant, and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

11.3.3 Wash the cadmium-copper with ammonium chloride solution several times to remove all the precipitated copper. The color of the cadmium so treated should be black.

11.3.4 Periodically the column may require a treatment of copper sulfate to coat the surface of the granules and extend the column life. Using a syringe and as little of the copper sulfate as possible (1 to 4 ml), force the solution across the cadmium, from both ends, until the black color returns. Too much copper sulfate will result in a brown precipitate forming inside the column which will interfere with flow and decrease the efficiency of the column.

11.4 Re-packing the Cadmium Column

WARNING: Cadmium is toxic and carcinogenic. Wear latex gloves when re-packing the column(s). After the column efficiency has dropped below 85% the column should be re-packed.

11.4.1 Open up both ends of the column, remove the end plugs. Hold the glass column over the waste cadmium container and gently tap and/or rinse the granules into the waste bottle with a stream of reagent water.

11.4.2 Rinse the column with reagent water. Replace one polyurethane foam plug and connect one plastic end with TEFLON tubing. Connect a short piece of tygon tubing between the threaded end of the column and a thistle tube bowl or small funnel.

11.4.3 Fill the thistle tube with ammonium chloride solution. Using a pipette bulb without the pipette holder, force the ammonium chloride down the column and out the TEFLON tube.

11.4.4 Add the copperized cadmium granules prepared earlier a few at a time to the thistle tube with a spatula and tap gently to solidify the cadmium in the column.

11.4.5 Remove the tygon tubing from the top and insert a foam plug. Re-attach the other end of the column with its threaded counterpart. Store until ready for use. When re-tightening the threaded plastic ends to the glass column check to be sure all threads are free of small pieces of cadmium or chipping can occur during tightening resulting in leaks. Use TEFLON tape on the threads to ensure a water tight seal.

11.4.6 The column must be activated before use. Running the standards through usually will do it. If standards fail the first time, re-standardize or run a 100 ppm nitrate sample thru the column.

11.5 Regeneration of the Cadmium column

The life of the column can be extended by gently running about 2-5 ml of the 2% copper sulfate soln through the column when off line by using a syringe. Alternatively, although not as good, the last sample tube can contain 2% copper sulfate.

12.0 Data Analysis:

Calculations for this procedure are performed by the FIA computer using a second or third order fit on the standards. Results will be reported in mg N/L and mg P/L to three decimal places.

If external standards are used a blank correction will need to be made.

13.0 Method Performance:

The following method detection levels (MDL) and precision were obtained by spiking seven samples of acid preserved reagent water with 0.02 ppm ortho phosphate and 0.04 ppm nitrate as KNO3 (see section 17).

Analyte#	Analyte	MDL(mg/L)	precision(σ)	bias	range mg/L
9415	total PO_4	0.004	0.00111	-9.1%	0.004 to 1
9595	total N	0.015	0.00464	-8.5%	0.015 to 2.5

14.0 Pollution Prevention:

A major source of pollution in this method is the Cadmium. All Cadmium after use will be collected and stored for proper disposal.

15.0 Waste Management:

Due to the hazardous nature of Cadmium its use should be minimized as much as possible. One way to do this is to use the prepared columns and try to regenerate them as many times as possible before discarding the waste into storage bottles. See Section 11.4.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 EPA (March 1983) Method 353.2 (colorimetric automated, cadmium reduction)

16.2 Lachat (Aug 1994) QuikChem Method No. 10-107-04-1-C (Nitrate/Nitrite)

16.3 Lachat (Oct 1994) QuikChem Method 10-115-01-1-E (Determination of Total Phosphorus by FIA colorimetry, acid persulfate Digestion method)

16.4 EPA Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11, 40 CFR Ch.1 d(7-1-94 Edition).

16.5 Persulfate Method (Proposed) 4500-N_{org} D.; Standard Methods for the Examination of Water and Wastewater, 19th Ed. 1995, 4-95. Amer. Public Health Association, 1015 Fifteenth St. NW Washington, DC 20005.

16.6 Nydahl, Folke. "On The Peroxodisulphate Oxidation of Total Nitrogen in Waters to Nitrate"; Water Research Vol 12, pp 1123 to 1130; 1978.

17.0 Tables, Diagrams, Flowcharts, Validation Data and Additional Information

Raw data to be saved includes the printout of the mg N/L for the samples along with the printing of the regression analysis of the standards. The electronic data file will also be kept for 3 years. After the data has been entered into the LIMS, the distribution sheet will also be kept. Completed results will be stored in the TKN/PO4 book.

Correction for efficiency of Cadmium column.

In normal real world samples the efficiency of the column will distort the standards and the samples equally and our nitrate + nitrite values would be very good because of the small amount of nitrite. In this digestion procedure however all nitrite will be converted to nitrate during the block phase of the analysis. However as noted earlier column efficiencies lower than 85% indicate the column needs attention.

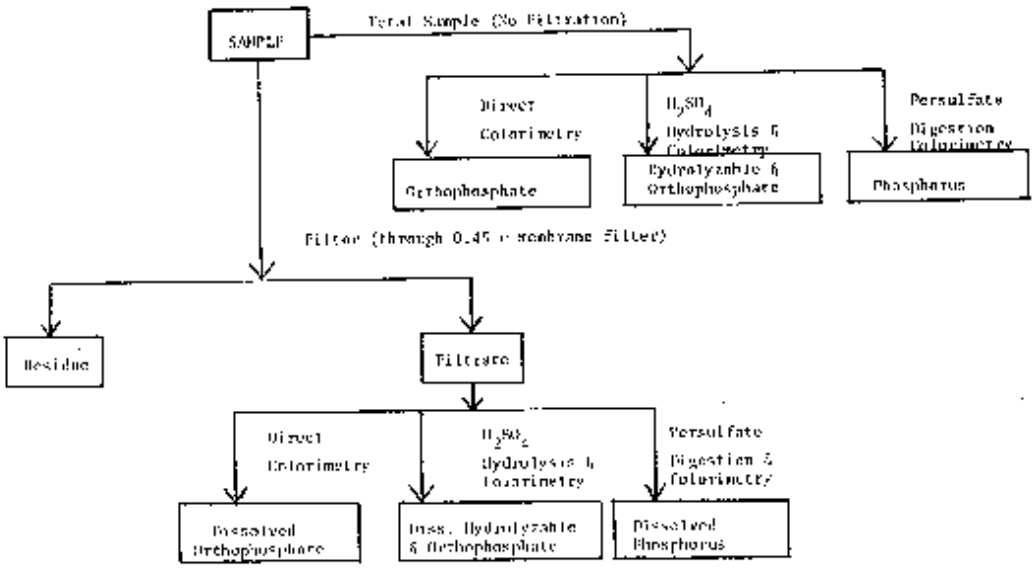
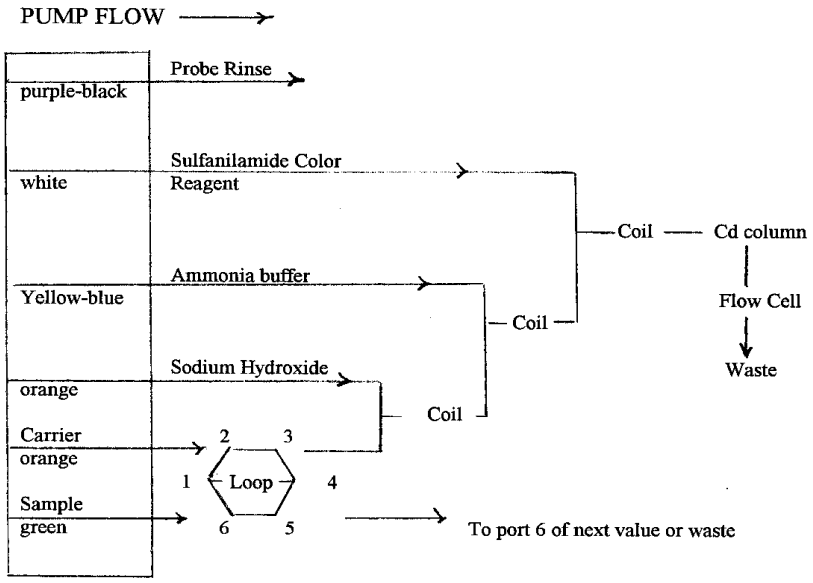


FIGURE 1. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

Nitrate/Nitrite Manifold Diagram



Notes

Sample loop = 190 cm of 0.022 inch inside dia PTFE tubing
Interference filter = 520 nm
Coils are 3/8 inch dia by 4 inch fully wrapped with 0.032 inch inside dia PTFE tubing
Pump speed = 35 (12.2 rpms)
Cadmium column is 3 mm X 250 mm threaded glass

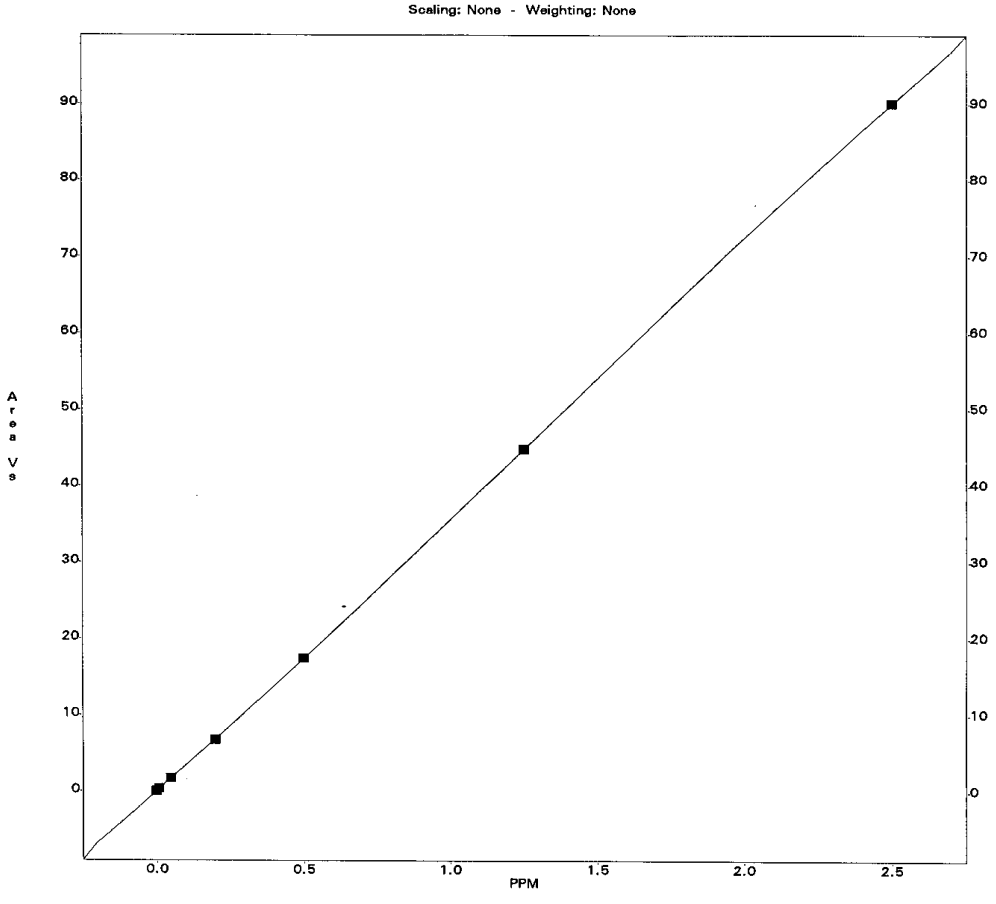
Total Nitrogen Calibration Curve

external standards

TKN (NO3)

Lvl	Area	PPM	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 3rd Poly
1	90136576	2.50	90136576					0.0	0.0	-0.0
2	44816448	1.25	44816448					0.0	0.0	0.0
3	17464126	0.50	17464126					0.0	0.0	-0.3
4	6796320	0.20	6796320					0.0	0.0	1.0
5	1711034	0.05	1711034					0.0	0.0	0.4
6	371715	0.01	371715					0.0	0.0	-3.3
7	51245	0.00	51245					0.0	0.0	

3rd Order Poly
Conc = 3.751e-025 Area³ - 5.411e-017 Area² + 2.957e-008 Area - 6.587e-004
r = 1.0000



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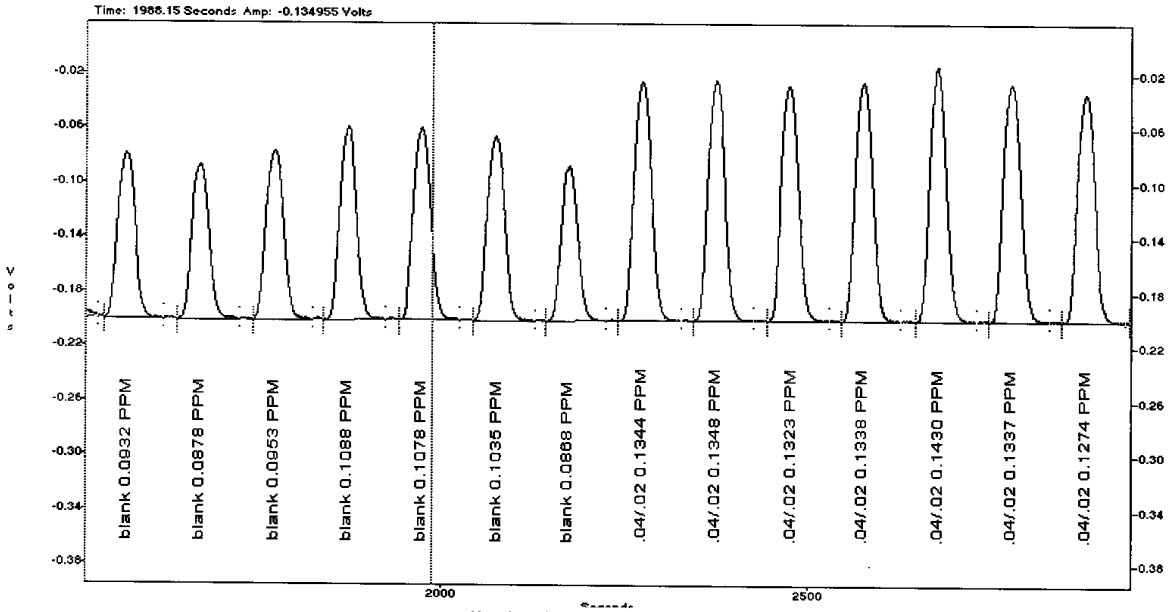
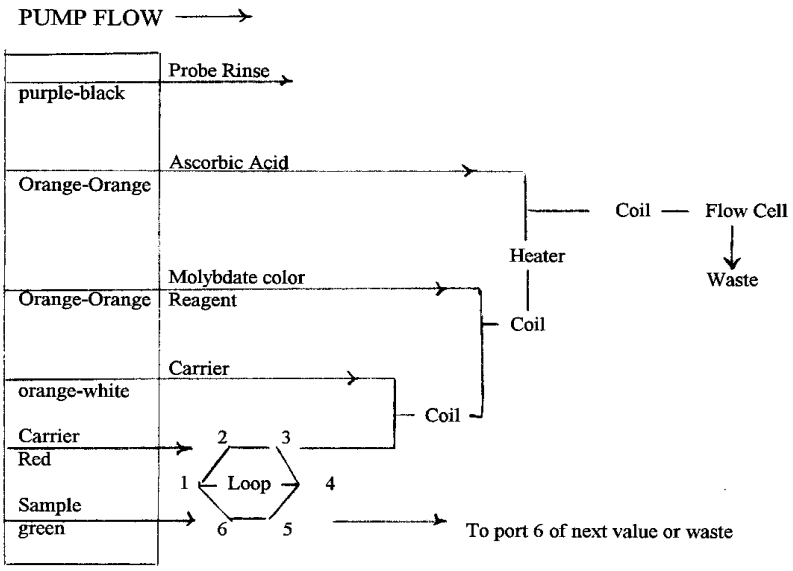
Nitrogen Detection Level



mean = spiked value - blank
= 0.134198 - 0.097601 = 0.036597 ppm
bias = -8.5 %
σ = 0.004639
MDL = (0.004639)(3.14) = 0.0146 ppm

Phosphate Manifold Diagram



Notes

Sample loop = 190 cm of 0.022 inch inside dia PTFE tubing
Interference filter = 880 nm
Coils are 3/8 inch dia by 3 inch fully wrapped with 0.032 inch inside dia PTFE tubing
Pump speed = 35 (12.2 rpms)
Heater set at 37 deg C.

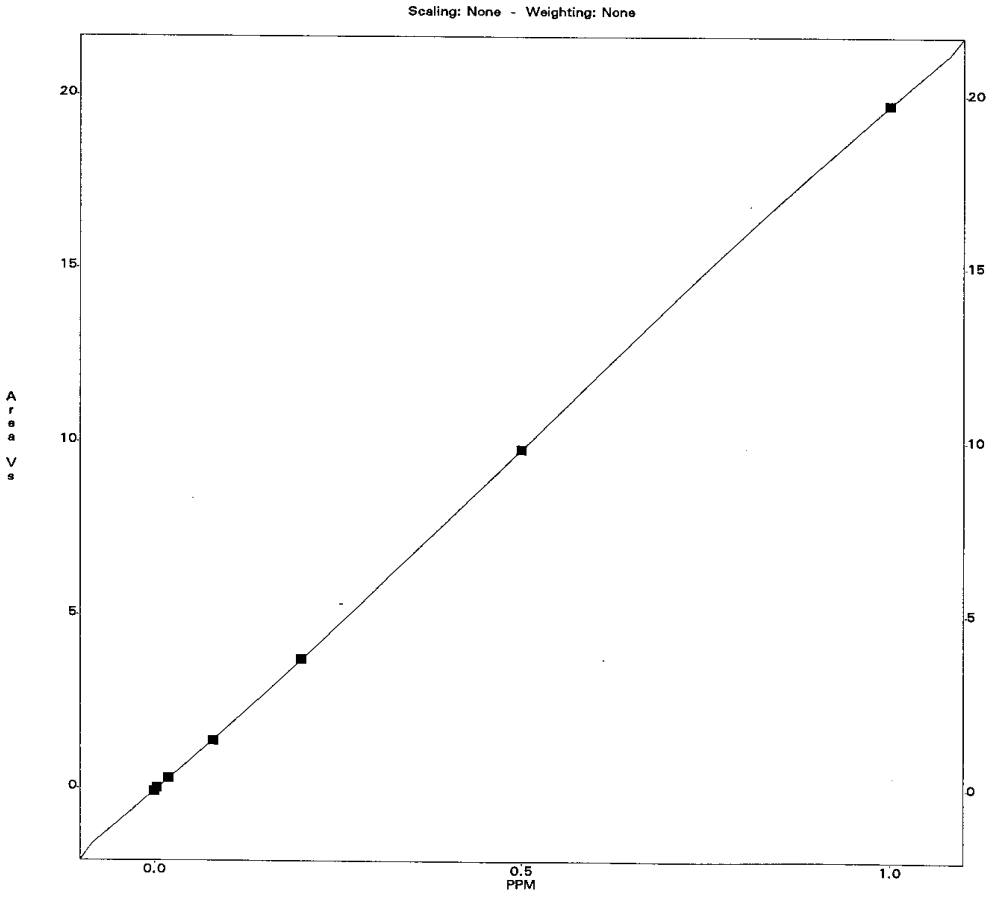
Total Phosphorus Calibration Curve

external standards

PO4

Lvl	Area	PPM	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 3rd Poly
1	19731268	1.000	19731268					0.0	0.0	-0.0
2	9797063	0.500	9797063					0.0	0.0	0.0
3	3750739	0.200	3750739					0.0	0.0	-0.5
4	1386845	0.080	1386845					0.0	0.0	2.2
5	305686	0.020	305686					0.0	0.0	-1.8
6	22054	0.004	22054					0.0	0.0	-23.8
7	-75437	0.000	-75437					0.0	0.0	

3rd Order Poly
Conc = 1.917e-023 Area³ - 5.806e-016 Area² + 5.448e-008 Area + 3.752e-003
r = 1.0000



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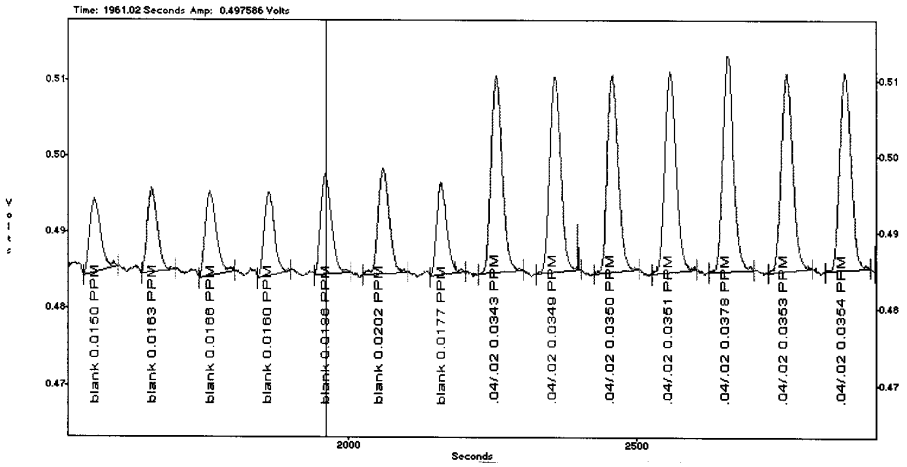
Phosphorus Detection Level



n = 7
mean = spiked value - blank
= 0.035388 - 0.017207 = 0.01818 ppm
bias = -9.1%
σ = 0.001111
MDL = (0.001111)(3.14) = 0.0035 ppm

Control charts for alkaline digestion of adenosine 5'-triphosphate